REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and following remarks.

Claims 4, 6, 9-10 and 14-22 have been cancelled without prejudice and replaced with new claims 23-27. New claim 23 corresponds to a combination of claims 14-16, 4, 6 and 9. New claims 24-27 correspond to claims 18-21, respectively. In claim 1, the method has been define to include step (c) of repeating said steps (a) and said step (b) to develop increased tolerance of said mutant *Escherichia coli* dnaQ49 strain to said drug until the tolerance is at least 1,000 times higher than that of wild type *Escherichia coli*. This requirement is supported, for example, by Table 1 on page 21 of the specification. Table 21 shows mutant dnaQ49 is tolerant to 3,000μg/ml and 6,000μg/ml of ampicillin, while wild type is tolerant to 3-6 μg/ml of the drug. Also, Table 3 shows the mutant dnaQ49 is tolerant to 500μg/ml of ofloxacin, while the wild type did not grow in the presence of 0.1 μg/ml of the drug. Tolerance to nalidixic acid and streptomycin are shown in Tables 4 and 5.

Turning to the Official Action, claims 4, 6, 9-10 and 14-22 were rejected under 35 USC 103 as being unpatentable over Fijalkowska et al. and Lin et al. in view of either Imamoto et al. or Iwaki et al. and further in view of Pan et al. This ground of rejection of respectfully traversed as applied to the wording of the new claims.

Claim 23 is directed to a novel method for establishing a mutant of *Escherichia coli* dnaQ49 strain having a tolerance to an antibiotic drug of at least 1,000 times higher than that of the wild type *Escherichia coli*. The method involves introducing a mutation into the genomic of *Escherichia coli* dnaQ49 strain by culturing it under a certain temperature. A mutant *Escherichia coli* dnaQ49 strain is then selected which is tolerant to the drug. These two steps are repeated to develop increased tolerance of the mutant to the drug until the tolerance is at least 1,000 times higher than the wild type tolerance. Moreover, step (b) is repeated under a higher concentration drug than in the prior step (b), and step (a) is repeated under the same concentration of the drug as in step (b) immediately therebefore. These features are not disclosed or suggested in the art, and are unexpectedly effective in establishing a high tolerance *E. coli* is a short period of time.

For a detailed description of the method, please see pages 18-20 of the specification.

The cited references do not disclose or suggest the claimed method either individually or in combination. The references fail to disclose or suggest step (c) of claim 23. The references fail to disclose or suggest that *E. coli* dnaQ49 strain may be established having a tolerance to an antibiotic drug of at least 1,000 times higher than the wild type.

The highest resistance concentration of ampicillin-resistant *Escherichia coli* which had been reported at the time of this invention was 1,500 μg/ml, i.e. a tolerance of only 250-500 times the wild type of 3-6 μg/ml and the resistance was due to a plasmid. When mutagenesis was carried out at 37°C without addition of ampicillin, it was not possible to obtain an ampicillin-resistant microorganism even by ten operations.

As a result of the claimed method, resistant microorganisms showing a resistance to ampicillin of unexpectedly high concentrations were able to be acquired within a short period.

In summary, it is respectfully submitted that the method of claim 23 could not have been expected from the combined teachings of the prior art. In addition, the mutants of claims 24-27 could not have been expected.

Favorable reconsideration and allowance is thus respectfully solicited.

Respectfully submitted,

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